Bacterial Cell Wall

Beneath the external structure as capsules, sheaths, and Flagella and external to the cytoplasmic membrane is the cell wall, a very rigid structure that gives shape to the cell. Its main function is to prevent the cell from expanding and eventually bursting because of uptake of water, since most bacteria live in hypotonic environments (i.e., environments having a lower osmotic pressure than exists within the bacterial cells). The rigidity of the wall can be readily demonstrated by subjecting bacteria to very high pressure or other severe physical conditions: most bacterial cells retain their original shapes during and after such treatments. To obtain isolated cell walls for analysis, bacteria usually must be mechanically disintegrated by drastic means, as by sonic or ultrasonic treatment or by exposure to extremely high pressures with subsequent release of pressure. The broken cell walls are then separated from the rest of the components of the disintegrated cells by differential centrifugation. Isolated cell walls, devoid of other cellular constituents, retain the original contour of the cells from which they were derived. Among the ordinary or typical bacteria (which are sometimes called eubacteria to distinguish them from the phylogenetically district group known as the archeabacteria), the wall of gram-negative species are generally thinner (10 to 15 nm) than those of gram-positive species (20 to 25 nm). The walls of gram-negative archeabacteria are also thinner than those of gram-positive arecheabacteria. Since the chemical composition of the walls of archeabacteria is quite different from that of eubacteria, wall thickness rather than chemical composition may be the major factor in the gram reaction.

The cell wall constitutes a significant portion of the dry weight of the cell; depending on the species and culture condition, it may account for as much as 10 to 40 present. Bacterial cell walls are usually essential for bacterial growth and division. Cells whose walls have been completely removed (i.e., protoplasts) are incapable of normal growth and division.

The cell wall is one of the most important parts of a prokaryotic cell for several reasons. Except for the mycoplasmas and some archeabacteria most bacteria have strong walls that give them shape and protect them from osmotic lysis. The cell walls of many pathogens have components that contribute to their pathogenicity. The wall can protect a cell from toxicity and is the site of action of several antibiotics.

After Christian gram developed the gram stain is 1884, it soon became evident that bacteria could be divided into two major groups based on their response to the gram-stain procedure. Gram-positive bacteria stained purple whereas gram-negative bacteria were coloured pink or red by the technique. The true structural difference between these two groups become clear with the advent of the transmission electron microscope. The gram-positive cell wall consists of a single 20 to 80nm thick homogeneous peptidoglycan or murein layer lying outside the plasma membrane. In contrast, the gram-negative cell wall is quite complex. It has a 1 to 3nm peptidoglycan layer coated by a 7 to 8 nm thick outer membrane. Microbiologists often call all the structures outside the plasma membrane the envelope. This includes the wall and structures like capsules when present.

Frequently a space is seen between the plasma membrane and the outer membrane in electron micrographs of gram-negative bacteria, and sometimes a similar but smaller gap is observed between the plasma membrane and wall in gram-positive bacteria, this space is called the periplasmic space or periplasm. Recent evidence indicates that the periplasmic space may be filled with a loose network of peptidoglycan. Possibly it is more a gel than a fluid –filled space. Size estimates of the periplasmic space in gram-negative bacteria range from 1nm to as great as 71 nm. Some recent studies indicate that it may constitute about 20 to 40% of the total cell volume (around 30 to 70nm), but more research is required to establish an accurate value. When cell walls are disrupted carefully or removed without disturbing the underlying plasma membrane, periplasmic enzymes and other proteins are released and may be easily studied. The periplasmic space of gram-negative bacteria contains many proteins that participate in nutrient acquisition-for example, hydrolytic enzymes attacking nucleic acids and phosphorylated molecules, and binding proteins involved in transport of materials into the cell. Gram-positive bacteria do not appear to have as many periplasmic proteins; rather, they recreate several enzymes that ordinarily would he periplasmic in gram- negative bacteria. Such recreated enzymes are often called exoenzymes.

The recently discovered archaeabacteria differ from other bacteria in many respects. Although they may be either gram positive or gram negative, their cell walls are distinctive in structure and chemical composition. The walls lack peptidoglycan and are composed of proteins, glycoproteins or polysaccharides.

Following this one view of the envelope, peptidoglycan structure and the organisation of gram-positive and gram-negative cell walls are discussed in more detail.

Function

1. It gives shape to the cell which is the characteristic of the bacterial species. The shape is genetically determined and given by the cell wall.
2. It gives protection to the bacterial cells against the destruction or lysis by osmotic shock. The structure without cell wall and the contents are cover by cell membrane then they are spherical and has no shape at all. Then it is called protoplast. Cell wall is a rigid cross-linked structure which is resistant to the effect of the huge osmotic pressure difference between the external and inside of the cell. So, they have no higher osmotic shock.

Structure and chemical composition

Peptidoglycan

For eubacteria, the shape-determining part of the cell wall is largely peptidoglycan (sometimes called murein), an insobluble, proteins, cross-linked polymer of enormous strength and rigidity. Peptidoglycan is found only in prokaryotes; it occurs in the from of a “bag-shaped macromolecule” outside the cytoplasmic membrane. Peptidoglycan differs somewhat in composition and structure from one species to another but it is basically a polymer of N-acetylglucosamine and N-acetylmuramic acid. L-alamine,(humans have only this L-form), D-alamine, D-glutamate, and a diamino acid LL-or mero-diaminopimlic acid an important/unique

feature of peptidoglycan, L-Lysine, L-osmithine, or L-diaminobutyric acid. It is important to realize that as though as peptidoglycan is, it is also in a dynamic state. That is, in order for the cell to grow and divide, portions of the peptidoglycan must continually be degraded by well-associated hydrolytic enzymes so that new polymer can be added.

Peptidoglycan or murein is an enormous polymer composed of many identical subunits. The polymer contains two sugar derivatives N-acetylglucosamine and N-acetylmuramic acid (the lactyl ether of N-acetylglucosamine), and several different amino acids, three of which –D-glutamic acid, D-alanine and meso-diaminopimelic acid – are not found in proteins. The peptidoglycan submit present in most gram-negative bacteria and many gram-positive ones as the name comprises is composed of peptides and glycan (a polysaccharide of glucose). The backbone of this polymer is composed of alternating N-acetylglucosamine and N-acetylmuramic acid residues. A peptide chain of four alternating D and L-amino acids is connected to the carboxyl group of N-acetylmuramic acid. Many bacteria substitute another diamino acid, usually L-lysine, in the third position for meso-diaminopimelic acid.

Chains of linked peptidoglycan submits are joined by cross-links between the peptides. Often the carboxyl group of the terminal D-alanine is connected directly to the amino group of diaminopimelic acid, but a peptide interbridge may be used instead. Most gram-negative cell wall peptidoglycan lacks the peptide interbridge. This cross linking results in an enourmous peptidoglycan sac that is actually one dense, interconnected network. These sacs have been isolated from gram-positive bacteria and are strong enough to retain their shape and integrity, yet they are elastic and somewhat stretchable, unlike cellulose. They also must be porous, as molecules can penetrate them**.**

Synthesis of Macromolecules : The Structure and Bio-synthesis of a cell-wall peptidoglycan

In all cells the major end products of biosynthesis are proteins and nucleic acids. However, there are other macromolecules peculiar to the prokaryotes which requires specialized biosynthetic processes. The utilization of energy in one of these processes is illustrated by the biosynthesis of bacterial cell-wall peptidoglycan. This particular biosynthetic process also serves as an example of how polymers are synthesized outside the membrane. Synthesis of cell-wall components is of interest because polymerization takes place outside the cell membrane by enzymes located on the membrane outer surface.

Structure of peptidoglycan

As discussed the rigid portion of a bacterial cell wall is a polymeric structure known as a murein, peptidoglycan, or mucopeptide. The walls of gram-positive bacteria contain a large proportion of peptidoglycan; those of gram-negative bacteria have a much smaller proportion.

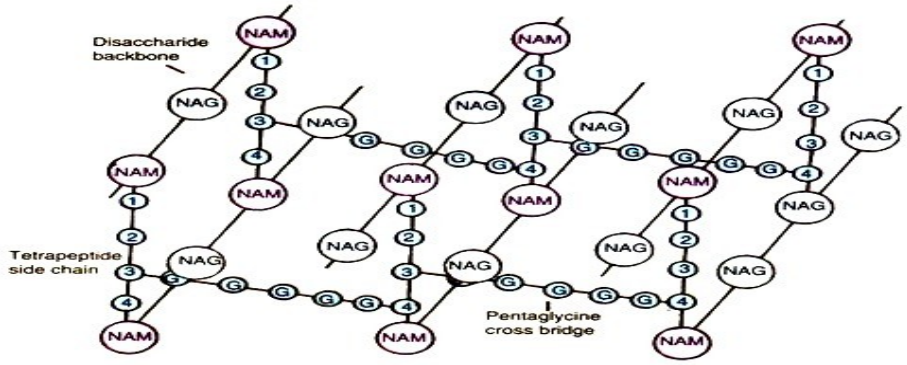


Fig :- General structure of peptidoglycans.

Peptidoglycans vary in their chemical composition and structure from species to species, but there are basic similarities. Peptidoglycans are very large polymers composed of three kinds of building blocks: 1)Acetylglycosamine (AGA Glc NAc),2) Acetylmuramic acid (AMA or Mur NAc), and 3)A peptide consisting of four or five amino acids of limited variety. Several of the amino acids exist in the D configuration, not usually found elsewhere in nature. A peptidoglycan can best be thought of as consisting of polysaccharide backbone chains composed of alternating units of AGA and AMA linked by β(1→4) bonds, with the short peptide chains projecting from the AMA units. Many of these peptide chains are cross-linked with each other, imparting great rigidity to the total structure. Some peptidoglycans differ in that the peptide chains may not be directly cross-linked to each other,being linked instead by another kind of peptide which forms a bridge between the terminal carboxyl group of one side chain with the free amino group of lysine or diaminopimelic acid (DPM or DAP) on the other side chain;,e.g.; in *Staphylococcus aureus* a bridge composed of five glycine molecules can link two muramic acid peptides together.

Activation of a peptidoglycan precursor

Escherichia coli can synthesize cell wall peptidoglycan when grown in a simple medium of glucose, ammonium sulfate, and mineral salts. One of the early steps in this synthesis is the formation of an activated derivative of AMA. This process requires energy at several paints and occurs in the cytoplasm. The activation of sugars, such as acetyl glucosamine, by the attachment of a uridine diphosphate (UDP) to form a sugar-UDP precursor is not peculiar to AMA but is a general method involved in the biosynthesis of many kinds of polysaccharides.

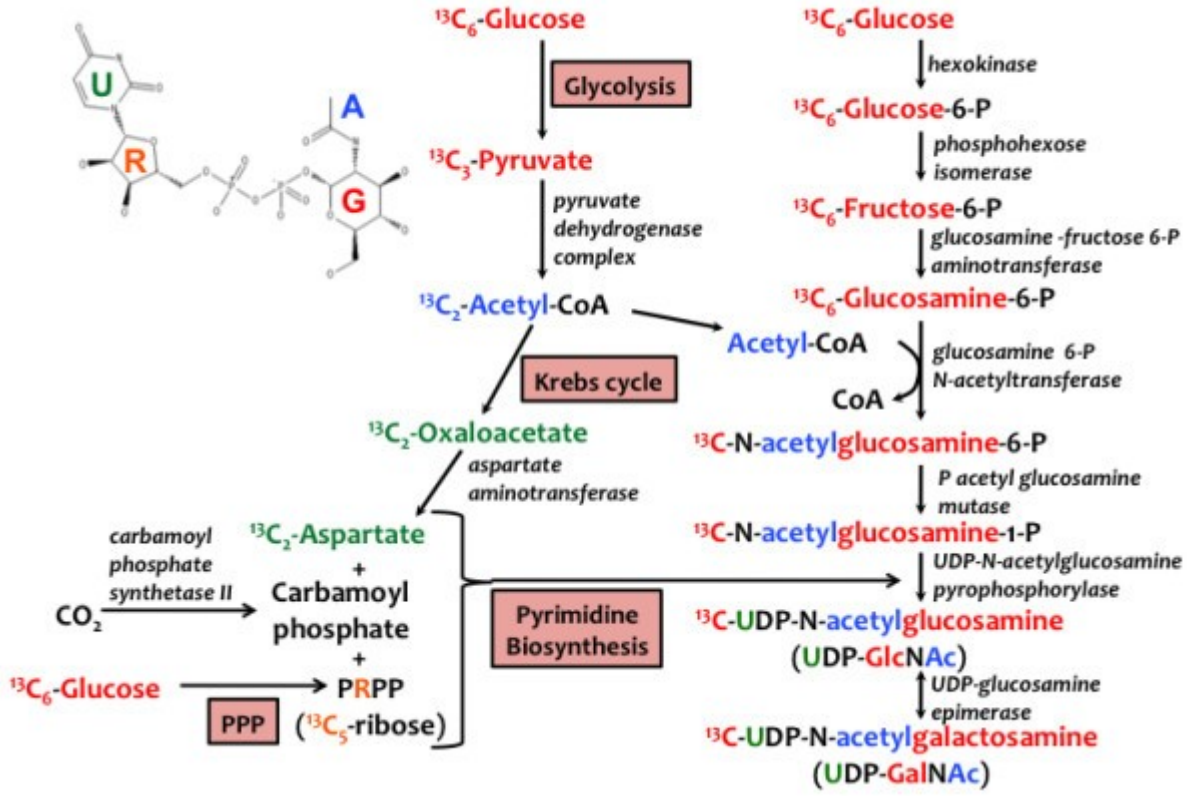


Fig :- Biosynthesis of acetylglucosamine-UDP and acetyl-muramic acid-UDP, key precursors in the synthesis of peptidoglycans.

This process takes place in cytoplasm. The high energy compounds are ATP, ADP, Pi, Acetyl CoA, CoA, UTP, PEP, NADPH and $NADP^+$.

Synthesis of Peptidoglycan

After formation of the activated AMA, the synthesis of peptidoglycan proceeds as follows:

1) Amino acids are sequentially to the AMA portion of the activated precursors to form a short pentapeptide chain. Ribosomes are not involved, but each amino acid addition required energy from the breakdown of ATP and the presence of Mg^{+2} or Mn^{+2} and a specific enzyme. These reactions occur in the cytoplasm.
2) The AMA-UDP processor is coupled to a membrane phospholipid called bactoprenol (indecaprenol phosphate).
3) The AGA couples with AMA of the AMA-UDP precursor. This reaction requires the activated form of AGA, that is the AGA-UDP derivative. In some organisms, the addition of bridging peptides takes place at this step. Reactions of steps 2 and 3 occur in the cell membrane.
4) The precursor, still linked to bactoprenol, is carried out of the cell though the cell membrane and is linked to a growing peptidoglycan chain in the cell wall. Peptide cross-linking may now occur, and the incorporation of the precursor into the growing peptidoglycan is thus completed. These reactions occur in the periplasm.

The synthesis of peptidoglycan illustrates the utilization of energy in joining together smaller molecules into larger ones. Note that all the energy needed for polymerization is used in the cytosolic (cytoplasmic) reactions in synthesizing the activated precursors. Other macromolecule synthesis requires a template which, acting like a tape provides information about the order in which the smaller pieces are assemble into larger ones, such processes include DNA synthesis (another piece of DNA is the template) and protein synthesis (a molecule of RNA serves as template).

The Mechanism of Gram-Staining

Although several explanations have been given for the gram stain reaction results, it seems likely that the difference between gram-positive and gram-negative bacteria is due to the physical nature of their cell walls. If the cell wall is removed from gram-positive bacteria they become gram negative. The peptidoglycan itself is not stained; instead, it seems to act as a permeability barrier preventing loss of crystal violet. During the procedure, the bacteria are first stained with crystal violet and next treated with iodine to promote dye retention. When gram positive bacteria then are decolorized with ethanol, the alcohol (or alcohol – acetone mixture) is thought to shrink the pores of the thick peptidoglycan. Thus, the dye-iodine complex is retained during the short decolorization step and the bacteria remain purple. In contrast, gram-negative peptidoglycan is very thin, not as highly cross-linked, and has larger pores. Alcohol treatment also may extract enough lipid from the gram-negative wall to increase its porosity further. For these reasons, alcohol more readily removes the purple crystal violet-iodine complex from gram-negative bacteria.

Wall of Gram-Positive Eubacteria

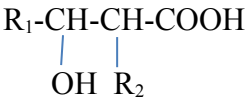
Gram-Positive bacteria usually have a much greater amount of peptidoglycan in their cell walls than do gram-negative bacteria; it may account for 50 percent or more of the dry weight of the wall of some gram-positive species, but only about 10 percent of the wall of gram-negative bacteria. Other substances may occur in addition to peptidoglycan.

1) For instance the walls of *Streptococcus pyogens* contain polysaccharides that are covalently linked to the peptidoglycan and which can be extracted without dilute hydrochloric acid.
2) Normally, the thick, homogeneous cell wall of gram-positive bacteria is composed primarily of peptidoglycan which often contains a peptide inter-bridge. However, gram-positive cell walls usually also contain large amounts of teichoic acids, polymers of glycerol or ribitol joined by phosphate groups. Amino acids such as D-alanine or sugars like glucose are attached to the glycerol and ribitol groups. The teichoic acids are connected to either the peptidoglycan itself or to plasma membrane lipids; in the latter case. They are called lipoteichoic acids. Teichoic acids appear to extend to the surface of the peptidoglycan, and because they are negatively charged, help give the gram-positive cell wall into negative charge. The functions of these molecules are still unclear, but they may be important in maintaining the structure of the wall. Teichoic acids are not present in gram-negative bacteria.

   The walls of *Staphlylococcus aureus* and *Streptococcus faecalis* contain teichoic acids –Acidic polymers of ribitol phosphate or glycerol phosphate which are covalently linked to peptidoglycan and which can be extracted with cold dilute acid. Teichoic acids bind magnesium ions, and there is some evidence that they help to protect bacteria from

thermal injury by providing an accessible pool of these cations for stabilization of the cytoplasmic membrane.

3) The walls of most gram-positive bacteria contain very little lipid, but share of *Mycobacterium, Cornybacterium*, and certain other genera are exceptions, being rich in lipids. There compounds have the following general structure :



Where R_1 and R_2 are long hydrocarbon chains. The ability of mycobacteria to exhibit acid-fast staining (i.e.; when stained, the cells cannot be decolorized easily despite treatment with dilute acids) is correlated with the presence of cell wall mycolic acids. A mycolic acid derivative called cord factor (trehalose dimycolate) is toxic and plays an important role in the disease caused by *C.diphthariae* and *M. tulerculosis*.

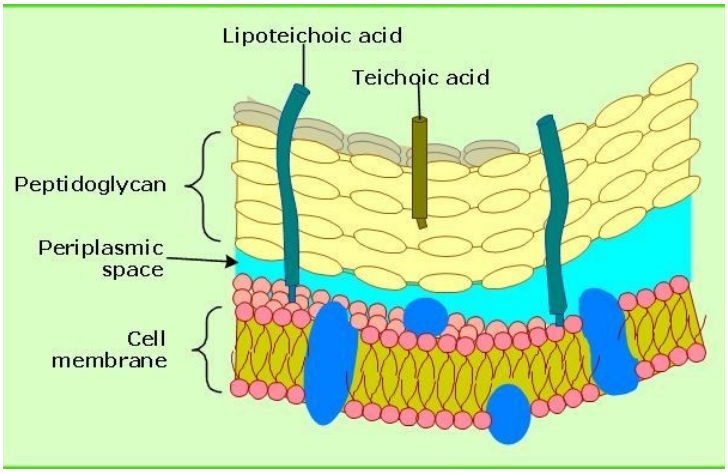


Fig: - The Gram-positive Envelope

4) Several protein structure also remain attached to this peptidoglycan layer eg. is M proteins as is present in case of *Streptococcus pyogens*. This is important for the pathogenesis of the particular bacteria. As the afforesaid one causes rheumatic fever, throat infections etc. All these structures give pathogenic potential for bacteria.

   Several antibiotics like Penicillin, is very important for the destruction of peptidoglycan. Thus such antibiotics that which are like penicillin like streptomycin etc. are used against gram-positive bacteria. It destroys the crossbridging in the peptidoglycan layer. But such antibiotics have no effect on human cells as they do not have peptidoglycan layer.

Different enzymes like lysozyme which is present in many secretions like saliva, tears and thus forms the first line defense mechanism it too destroys the peptidoglycan layer. But it should be kept in mind that no enzyme or antibiotics can restrict the transport across the gram-positive bacterial cell wall as it is very porous. It only dehydrating agents like alcohol which cannot dissolve it, but dries it reducing the pore size thus, the porosity is decreased. This is the reason why in gram-staining the dye-iodine complex is not washed out of the cell and it is stained purple.

But the defect of this process is that, as these agents destroy the peptidoglycan layer only, it is changed to protoplast, i.e.; no cell wall and it behaves like the gram-negative bacteria.

Gram-negative cell walls

The walls of the gram-negative bacteria are more complex than those of gram-positive bacteria. The walls of gram-negative bacteria have a comparatively low peptidoglycan content, seldom exceeding 5 to 10 % of the weight of the wall. The location of the peptidoglycan layer in this type of wall was first established by W. Weidel and his collaborators, for walls of *Escherichia coli*. In *E. coli*, it is about 1nm thick and contains only one or two layers or sheets of peptidoglycan. As mentioned earlier, the peptidoglycan may be in the form of a gel matter than a compact layer. They showed that the peptidoglycan constitutes the inner most layer of the multi layered wall and can be isolated as a very thin sac that retains the form and shape of the original cell, other wall components have been stripped off it by appropriate treatments.

The peptidoglycans of gram-negative bacteria characteristically display a rather low degree of cross-linkage between the glycan strands: many of the peptide chains are not cross-linked. The thickness of the peptidoglycan layer of the wall varies somewhat in different groups of gram-negative bacteria. Calculation suggest that in many gram-negative organisms it is a monomolecular (or at most bimolecular) layer.

There are several other structure present in gram- negative cell wall than is present in gram positive one. These additional structures are important for the additional stability of the gram-negative bacteria. They confer together with the peptidoglycan, protection, stability and strength to the cell wall. The most interesting difference is the presence of an outer membrane that surround a thin underlying layer of peptidoglycan.

The outer membrane

Superimposed on the thin murein sac characteristic of gram-negative bacteria is an outer layer that has the width and fine structure typical of a unit membrane. This layer, the outer membrane, has some chemical and physical proteins in common with the cell membrane, and others that are quite different. Like the cell membrane it is a lipid bilayer containing phospholipids and proteins, but in addition it contains large amounts of a unique lipid, lipopolysaccharide (LPS), which replaces, probably completely phospholipids in the outer leaf of this unique structure. Although chemically quite different from a phospholipids, LPS has physical properties that are sufficiently similar so that it can participate in forming a membrane; one end of the molecule is hydrophobic and the other is hydrophilic; the hydrophobic end becomes inserted in the membranes hydrophobic core and the hydrophilic end is on the outer surface.

The lipopolysaccharides are complex molecules with MW over 10,000 that vary widely in chemical composition, both within and between gram-negative groups. Most work on their structure has been conducted on the forms present in the *Salmonella* group. (*Salmonella typhimurium* mainly).

LPS is composed of three distinct regions; lipid A, the R core region/ core polyvaccharide, and the O side chain/ O antigen. Lipid A, the hydrophobic membrane-anchoring region of LPS, rather than carrying the two fatty acid residence typical of a phospholipid has six or seven attached to a phosphorylated glucosamine dimer. Unlike those in phospholipids, all the fatty acids in lipid A are saturated. Some are attached directly to the glucosamine dimer and others are esterified to the 3-hydroxy fatty acids that are characteristically present. Attached to

the 6 position of one glucosamine residue in lipid A is the R core oligosaccharide a short chain of sugars, which include two unusual ones, 2-keto-3- dcoxyoctonoic acid (KDO) and heptose. In *Salmonella,* it is constructed of ten sugars, many of them unusual in structure. The R core in turn bears the hydrophilic O side chain, likewise composed of sugars. It is much longer than the R core, being composed of many repeating tetra-or pentasaccharide units. The elucidation of this structure depended heavily on the availability of mutants, each blocked at a particular point in LPS biosynthesis. Biosynthesis of LPS is strictly sequential, starting with lipid A from which the ohigosaccharide is built by successive sugar additions, the O side chain being added last. The O side chain extend like whisks from the membrane surface into the surrounding medium. Many of the serological properties of gram-negative bacteria are attributable to O antigens; they can also serve as receptors for bacteriophage attachment. It has several peculiar sugars and varies in composition between bacterial strains. Although O side chains are readily recognized by host antibodies, gram-negative bacteria may thwart lost defenses by rapidly changing the nature of their O side chains to avoid detection. Antibody interaction with the LPS before reaching the outer membrane proper may also protect the cell wall from direct attack. The O antigen can be divided into different serological groups according to the type of Ab formation triggered in the serum. Thus it is important for its virulence. The LPS has tonic properties and is also known as endotoxin. The innermost region, consisting of lipid A and three residues of KDO, appears to be essential, but the rest of the molecule is dispensable.

The peptidoglycan layer of the wall bears a small (MW=7,200) specific type of lipoprotein, termed murein lipoprotein, which forms an anchoring bridge to the outer membrane. The C-terminus of this protein is a lysine residue which is peptide bonded to an amino group of a meso-diamino-pimelic acid residue that is not cross-linked in the peptidoglycan layer. At the other end (the N-terminus) of the protein is a cysteine residue to which fatty acids are attached : one is attached in an amide linkage to the terminal amino group, and two more are esterified to a glycerol residue which is attached by sulfur ether linkage to the cysteine.

The resulting brush like structure composed of the hydrophobic chains of these fatty acids becomes inserted into the inner leaf of the outer membrane, there by anchoring it to the peptidoglycan layer.

The physiological significance of the outer membrane is threefold: (1) it forms the outer limit of the periplasm, the region between the two membranes that contain a set of digestive enzymes. (2) It presents an outer surface with strong negative charge which is important in evading phagocytosis and the action of complement and (3) It provides a permeability barrier and there by increased resistance to a number of toxic agents. Among these are host defense agents like lysozyme, beta-lysin, and leucocyte protein, which are quite toxic to gram-positive bacteria which lack an outer membrane. Destructive agents in the mammalian digestive tract, such as bile salts and digestive enzymes and a variety of antibiotics, are also excluded by the outer membrane.

The LPS is important for several reasons other than the avoidance of host defences. Since the core polysaccharide, usually contains charged sugars and phosphate, LPS contributes to the negative charge on the bacterial surface. Lipid A is a major constituents of the outer membrane, and the LPS helps stabilize membrane structure. Further-more lipid A often is toxic; as a result ,

the LPS can act as an endo toxin and cause some of the symptoms that arise in gram-negative bacterial injections.

Thus the outer membrane is the outermost layer of the cell wall. Capsule etc.may be present above it but they are not a part of cell wall structure. Because of this membrane, the walls of gram negative bacteria are rich in lipids (11 to 22 percent of the dry weight of the wall), in contrast to those of gram-positive bacteria. This outer membrane serves as an impermeable barrier to prevent the escape of important enzymes, such as those involved in cell wall growth, from the space between the cytoplasmic membrane and the outer membrane (periplasmic space). The outer membrane also serves as a barrier to various external chemicals and enzymes that could damage the cell. As most of the enzymes are protein in nature thus it is hydrophilic, or water soluble or polar. Whereas the outer membrane is rich in lipid and thus is hydrophobic, thus it has a repelling tendency towards the polar substance. But the lipid nature substance can easily pass through it which water and hydrophilic substances cannot. For example, the walls of many gram-positive bacteria can be easily destroyed by treatment with an enzyme called lysozyme. Which selectively dissolves peptidoglycan; however gram-negative bacteria are refractory to this enzyme because large protein molecules cannot penetrate the outer membrane. Only if the outer membrane is first damaged, as by removal of stabilizing magnesium ions by a chelating agent, can the enzyme penetrate and attack the underlying peptidoglycan layer.

A most important outer membrane function is to serve as a protective barrier. It prevents or slows the entry of bile salts, antibiotics, and other toxic substance that might kill or injure the bacterium. Even so, the outer membrane is more permeable than the plasma membrane and permits the passage of small molecules like glucose and other monosaccharides. This is due to the presence of special porin proteins. Three porin molecules cluster together and span the outer membrane to form a narrow channel though which molecules smaller than about 600 to 700 daltons can pass. Larger molecules such as vitamin B_{12} must be transported across the outer membrane by specific carriers. The outer membrane also prevents the loss of constituents like periplasmic enzymes.

Although impermeable to large molecules such as proteins, the outer membrane can allow smaller molecules, such as nucleosides, oligasaccharides, peptides and amino acids, to pass across. This is accomplished by means of channels in special proteins called porins, which span the membrane. The various porins are specific for different kinds or classes of small molecules, and some can even allow certain essential large molecules to penetrate, such as vitamin B_{12}. Many porins also serve as receptors for attachment of bactteriophages and bacteriocins. The porins are thus a type of integrated protein which is specialized for various ions and water. The ions channels formed by the porins have their open state and closed state regulated by the pressure gradient or difference in the osmotic pressure on both the sides of the cell wall, i.e.; inside and outside the cell and by electrical gradient.

Of course the outer membrane cannot present a barrier to all substance in the environment because all cellular nutrients most pass through it. Permeability of the outer membrane to nutrients is provided in part by proteins collectively termed porins which, in

aggregates generally of three, form cross-membrane channels through which certain small molecules can diffuse. A variety of different porins are present in the outer membrane. They vary with respect to the size of the channel they form and the environmental conditions that stimulate their synthesis. For example, in *E. coli* the pore formed by Omp F (outer membrane protein F) is slightly larger than the one formed by Omp C. The synthesis of Omp F is repressed by elevated temperature (>37°c) and by growth in a medium of elevated osmotic pressure. The physiological rationale for this regulation of the synthesis of Omp F is presumed to be a mechanism of reusing whether the cell finds itself within a eucaryotic host or in an external environment. In the latter, usually cooler environment, the concentration of substrate is typically quite low; this necessitates the presence of larger pores formed by Omp F to allow diffusion of substrate molecules to occur at a greater rate, because rate of diffusion is proportional to the product of concentration difference across the membrane and cross-sectional area of the pore. Within a host where the concentration of substrates is typically much higher, the larger pore is unnecessary and even detrimental because antibacterial substance present in the host can enter more readily through these larger pores.

In addition to the nonspecific channels formed by porins, the outer membrane contains a variety of channels formed by other proteins that exhibit a remarkable specificity. For example, the channel sometimes called the maltoporin, formed by the inducible Lam B protein, specifically allow the diffusional entrance of the disaccharide maltose and maltodextrans into the cell. Maltotriose diffuses through these channels at 100 times the rate of the similar-sized trisaccharide, raffinose. Presumably, proteins that bind tightly to a specific substrate are associated with these channels, thereby conferring specificity on them.

In addition to the channel- forming proteins, a protein termed Qmp A is quite abundant in the outer membrane. Its specific role has not been clearly defined, but mutant strains that lack it produce a more fragile outer membrane, so we assume that Qmp A contributes in some way to the membranes structural integrity.

Although proteins constitute about half the mass of the outer membrane, until recently it was assumed that the member of different types of proteins located there was quite limited. Now it is clear that a large variety of different proteins are present in small quantities. With few exceptions, proteins in the outer membrane are not found in the cytoplasmic membrane.

The molecular basis of a remarkable property of the outer membrane that distinguishes it from other membrane, namely its impermeability to hydrophobic molecules is not yet understood, but this property accounts for resistance to certain dyes (eg. eosin, methylene blue and brilliant green) that are used in certain selective media.

The most abundant membrane protein is Braun’s lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The outer membrane and peptidoglycan are so firmly linked by this lipoprotein that they can be isolated as one unit.

One of the questions posed by the structure of gram-negative cell walls is; How can water- insoluble, liphophilic substances such as LPS pass from their place of synthesis within the cytoplasm and cytoplasmic membrane across a waterly periplasmic space to be inserted into the outer membrane? A likely explanation has been provided by the discovery of numerous adhesions or points of direct contact between the two membranes. These adhesions seem to be the export sites for newly synthesized LPS and porins, and they are also the sites at which pili and flagella are made.

Macromolecular Surface Array

The cell walls of some bacteria, both gram-negative and gram-positive are covered by a mosaic layer of protein subunits. The functions of these mosaic layers are not well understood, but at least one function is to protect gram-negative bacteria against attack and penetration by other small, predatory bacteria known as bdellovibrios.

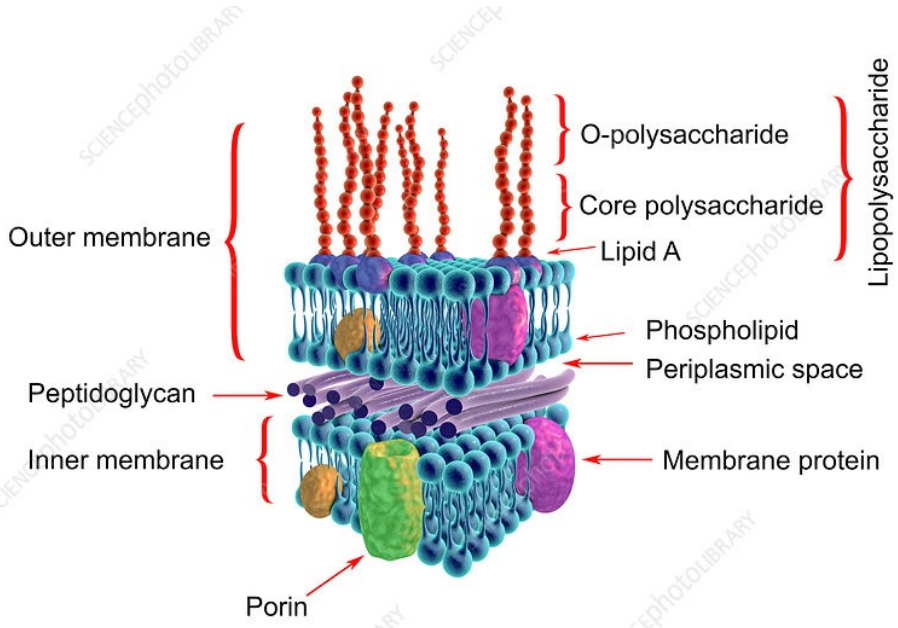


Fig: - The gram-negative Envelope

The cell wall and Osmotic protection

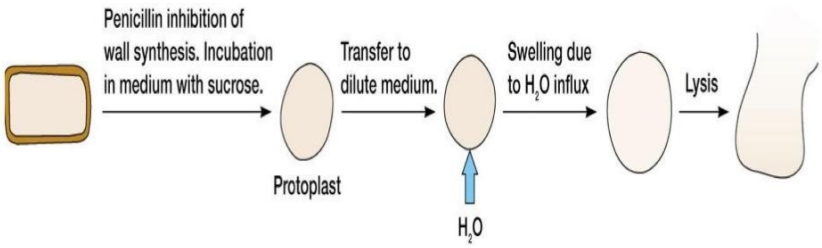
The cell wall is usually required to protect bacteria against destruction by osmotic pressure. Solutes are much more concentrated in bacterial cytoplasm than in most microbial habitats, which are hypotonic. During osmosis, water moves across selectively permeable membranes such as the plasma membrane from dilute solutions (higher water concentration) to more concentrated solutions (lower water concentration). Thus, water normally enters bacterial cells and the osmotic pressure may reach 20 atmospheres or 300 pounds/square inch. The plasma membrane cannot withstand such pressures and the cell will swell and lyse, or be physically disrupted and destroyed, without the wall that protects it by resisting cell swelling. Solutes are

more concentrated in hypertonic habitats than in the cell. Thus, water flows outward and the cytoplasm shrivels up and pulls away from the cell wall. This phenomenon is known as plasmolysis and is useful in food preservation because many microorganisms cannot grow in dried foods and jellies as they cannot avoid plamolysis.

The importance of the cell wall in protecting bacteria against osmotic lysis is demonstrated by treatment with lysozyme or penicillin. The enzyme lysozyme attacks peptidoglycan by hydrolyzing the bond that connects N-acetylmuramic acid with carbon four of N-acetylglucosamic. Penicillin inhibits peptidoglycan synthesis. If bacteria are incubated with penicillin in an isotonic solution, gram positive bacteria are converted to protoplasts that continue to grow normally when isotonicity is maintained even though they completely lack a wall. Gram-negative cells retain their outer membrane after penicillin treatment and are classified as spheroplasts because some of their cell wall remains. Protoplasts and spheroplasts are osmotically sensitive. If they are transferred to a dilute solution, they will lyse due to uncontrolled water influx.

Although most bacteria require an intact cell wall for survival, some have none at all. For example, the mycoplasmas lack a cell wall, yet often can grow in dilute media or terrestrial environments because their plasma membranes are stronger than normal. The precise reason for this not known, although the presence of sterols in the membranes of many species may provide added strength. Without a rigid cell wall, mycoplasmas tend to be pleomorphic or variable in shape.

The L forms (named after the Lister Institute in London where they were discovered) also lack cell walls. The loss may be complete or partial (some have a defective wall), and the parent organism may be either gram positive or gram negative. They are pleomorphic like mycoplasmas and continue to reproduce. These organism can arise through spontaneous mutations or from treatments such as growth in isotonic or hypertonic media containing penicillin. If all traces of the peptidoglycan disappear, bacteria cannot re-synthesize it because preexisting wall is necessary to construct new peptidoglycan. The L forms which cannot revert back to normal are bacteria in which the primer for peptidoglycan synthesis has been either eliminated or modified by penicillin treatment. In this case, the L form may be stable; (stable L form), that is, it may continue to grow and reproduce after the penicillin treatment has ceased. All unstable L forms, and certain stable ones, still contain muramic, but the concentration is relatively low (about 10-15% of its concentration in normal cells). Furthermore, the muramic acid is in an unusual chemical state, being readily extractable with dilute acid, whereas the muramic acid in a normal cell is not. Other L forms sometimes synthesize a wall again. L forms retain O antigens and are still susceptible to infection by phages for which the receptors are contained in the outer wall layer. These L forms were first identified in *Streptobacillus moniliforms* who normally are rod shaped. L forms are not closely related to mycoplasmas and should not be confused with them.



- Protoplast – cell completely lacking cell wall
- Spheroplast – cell with some cell wall remaining

Fig: - Protoplast formation